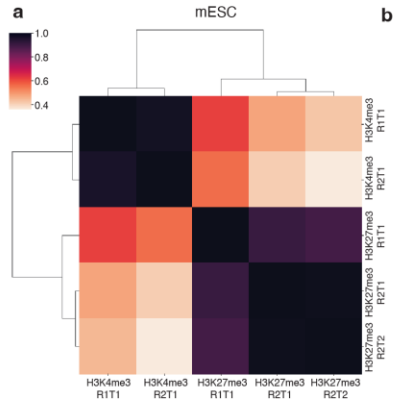
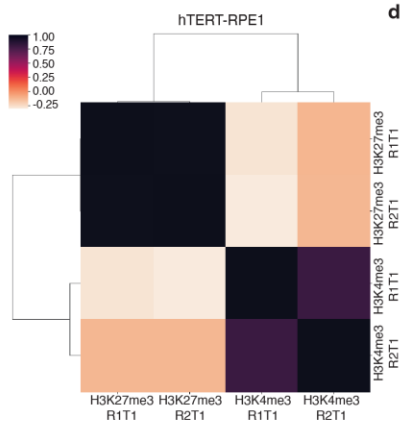
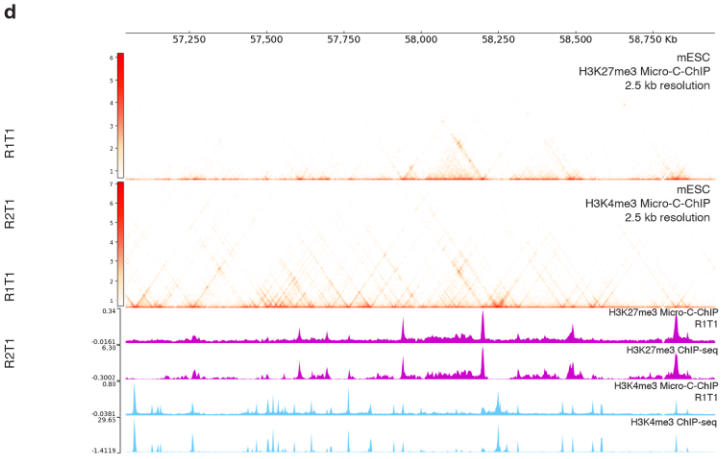
Supplementary Information

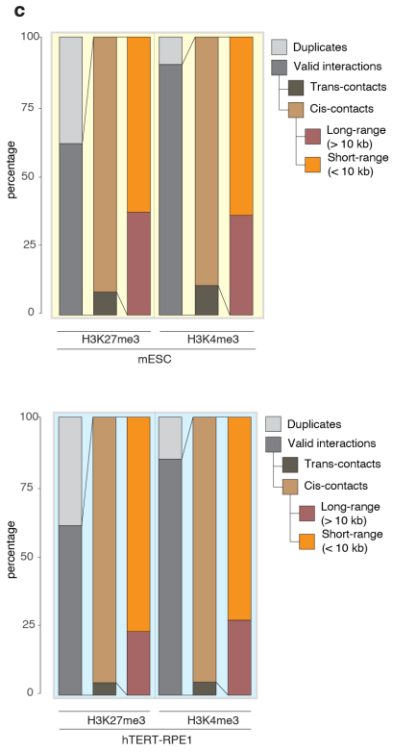


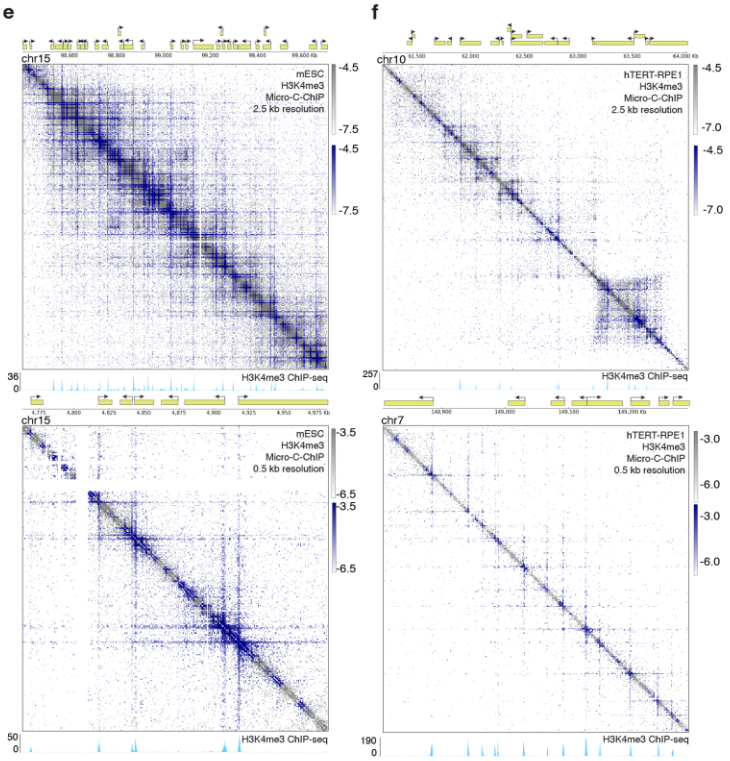


b

Cell type	Histone mark	Replicate	Total reads	Mapped reads	Uniquely mapped reads		Unique *cis* contacts, short-range (< 10 kb)	
					Read count	% mapped	Read count	% unique
mESC	H3K4me3	R1T1	226314129	145166938	122809286	84.60	58274283	52.55
		R2T1	336995737	241340104	225847218	93.58	68494855	69.68
	H3K27me3	R1T1	250335602	148534797	57774482	38.90	27613320	52.20
		R2T1	267859856	173898257	116620950	67.06	42086952	63.90
		R2T2	314904195	202052170	150558580	74.51	51220744	65.98
hTERT-RPE1	H3K4me3	R1T1	275429571	198587847	168055026	84.63	42949587	74.44
		R2T1	165503905	122316809	105771249	86.47	31336890	70.37
	H3K27me3	R1T1	276637770	197860070	87484140	44.22	18459344	78.90
		R2T1	186841875	132709915	115129939	84.75	28097964	75.59

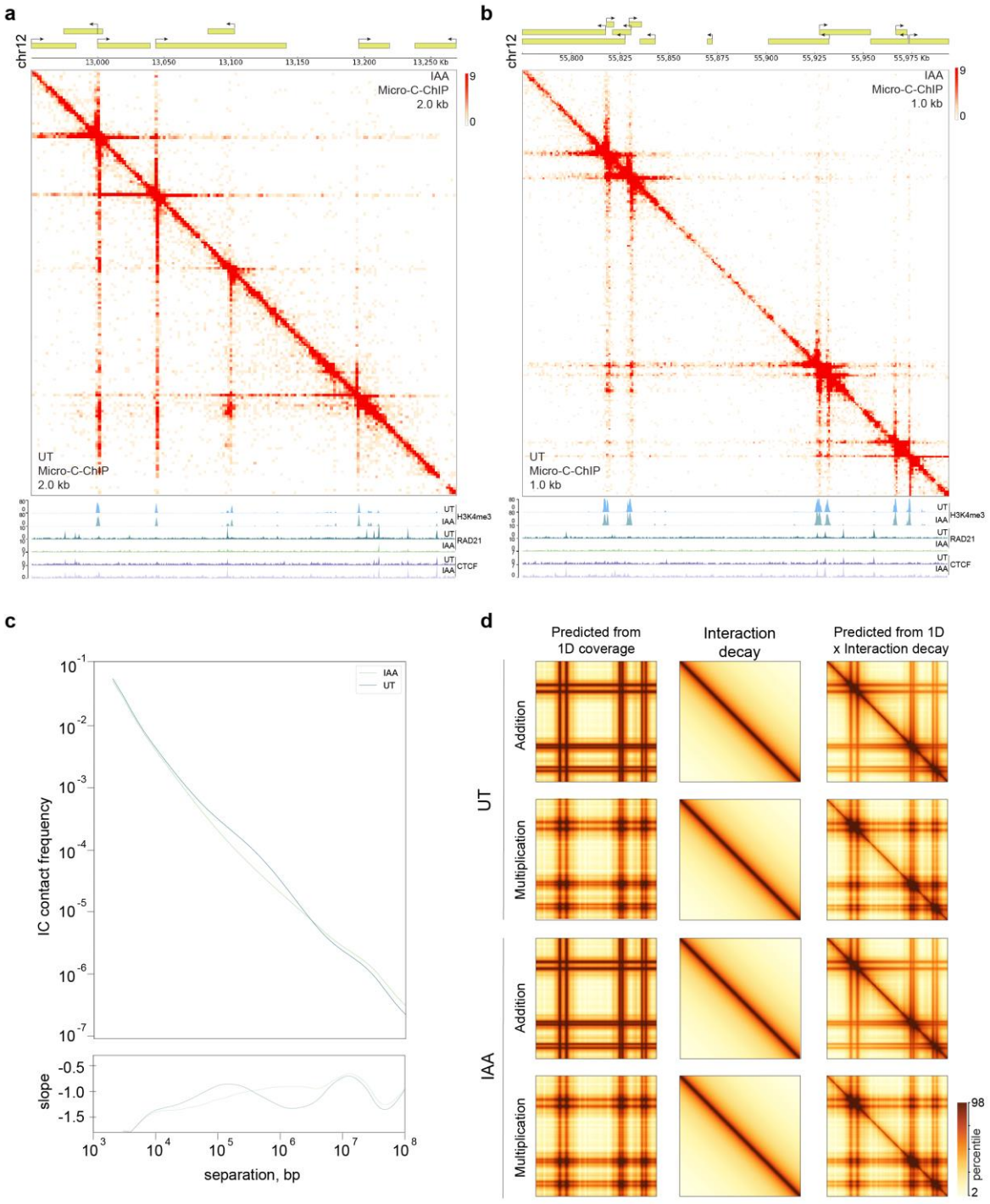






Supplementary Fig. 1. Micro-C-ChIP reproducibly captures highly precise histone mark-specific interactions in different cell types.

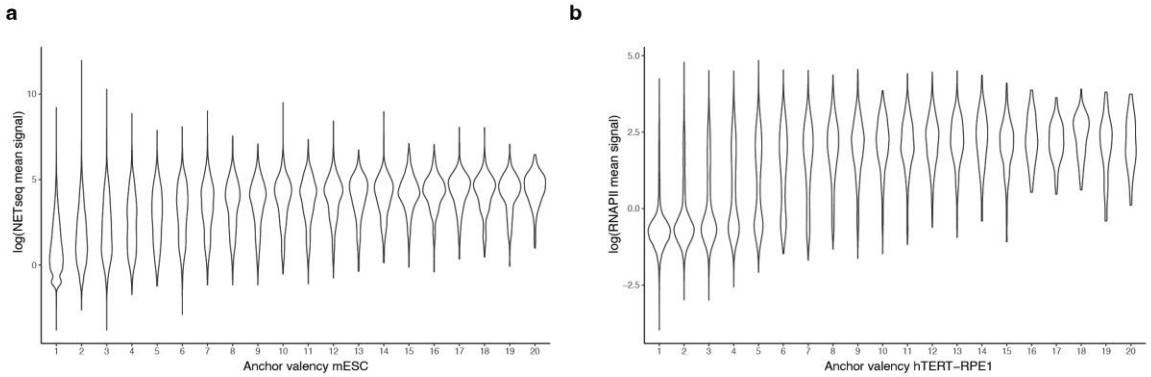
a, Reproducibility scores heatmaps for all replicates across both H3K4me3- and H3K27me3-enriched Micro-C-ChIP datasets in mESC (top) and hTERT-RPE1 (bottom). Reproducibility scores were determined using HiCRep at 10 kb resolution. **b**, Sequencing statistics of all Micro-C-ChIP replicates. The numbers of the total and mapped sequencing reads, unique reads and short-range (< 10 kb) unique *cis*-reads are shown. R1 and R2 indicate biological replicates, T1 and T2 - technical replicates. **c**, Sequencing statistics of the mESC (top panel) H3K27me3 and H3K4me3 and hTERT-RPE1 (bottom panel) H3K27me3 and H3K4me3 Micro-C-ChIP datasets. **d**, Unbalanced interaction heatmaps of merged H3K27me3 (top) and H3K4me3 (bottom) Micro-C-ChIP mESC datasets at different resolutions. CPM-normalized Micro-C-ChIP signal tracks for single replicates (c) and ChIP-seq signal tracks for the corresponding histone mark (c and d) are shown below the contact maps. **e**, **f**, Color-coded input-normalized interaction heatmaps of mESC (e) and hTERT-RPE1 (f) Micro-C-ChIP at 2.5 kb (top panel) and 0.5 kb (bottom panel) resolutions.



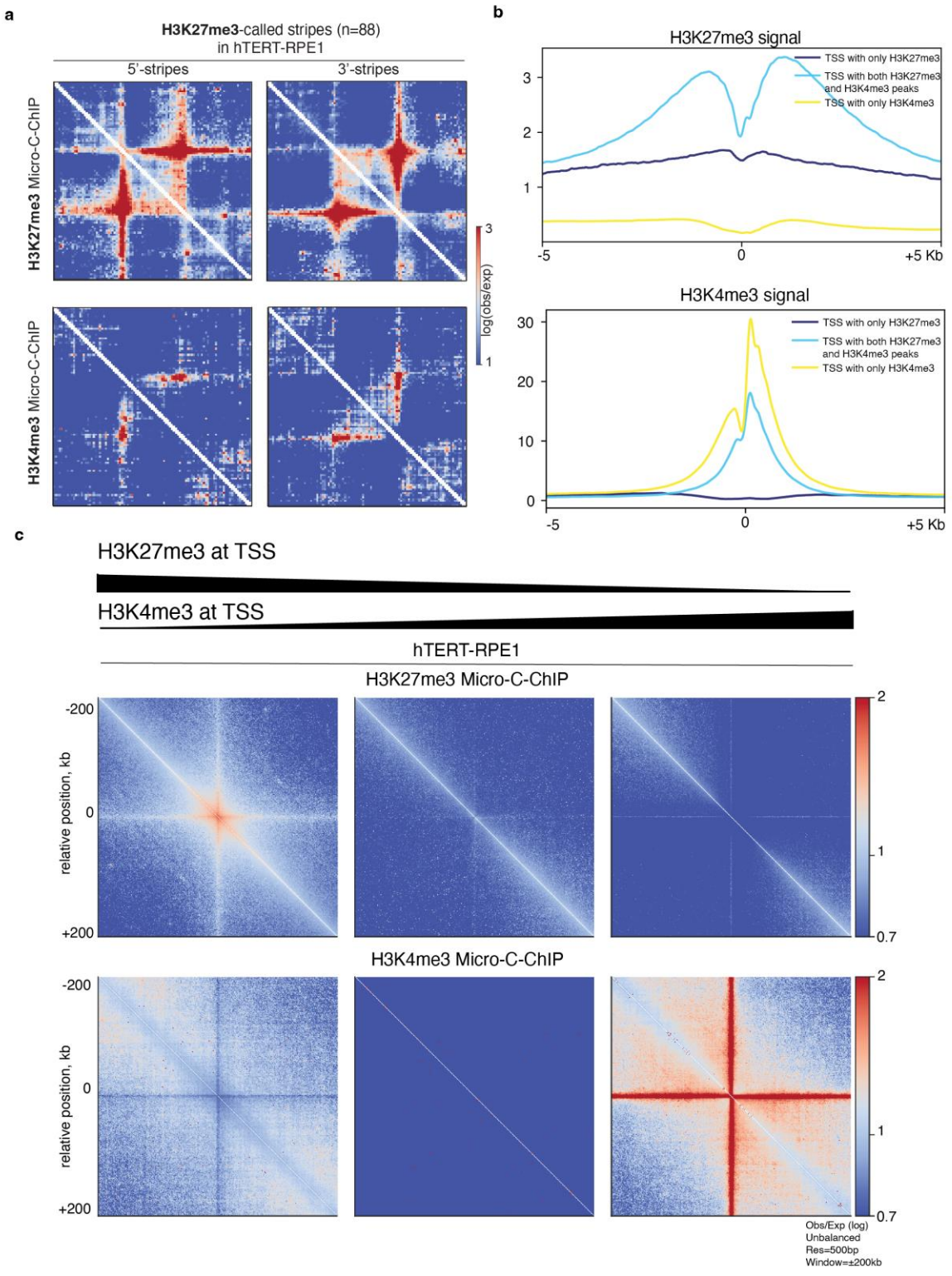
Supplementary Fig. 2. Micro-C-ChIP resolves the loss of H3K4me3-marked interactions upon RAD21 degradation, while it is not driven by the function of interaction decay.

a, b, Unbalanced interaction heatmaps comparing untreated (UT) (bottom-left triangle) and IAA-treated (IAA) (top-right triangle) HCT116-RAD21-mAC H3K4me3 Micro-C-ChIP at 2- (a) and 1-kb (b) resolutions. ChIP-seq signal tracks for H3K4me3, RAD21, and CTCF are shown below the contact maps. **c**, P(s) plot (top) and its derivative (bottom) for UT and IAA-treated cells. **d**, Heatmaps showing predicted interactions based on addition (upper panel) and multiplication (lower panel) of 1D signal values of UT and IAA Micro-C-ChIP datasets at the genomic region from a (chr12: 55,774,428 - 55,994,269). To assess the contribution of interaction decay, the 1D signal-based contact maps were multiplied by the expected decay curve. The

prediction results in nearly identical heatmaps for UT and IAA conditions, indicating that if interaction decay alone dictated changes upon RAD21 depletion, no differences would be observed upon RAD21 depletion

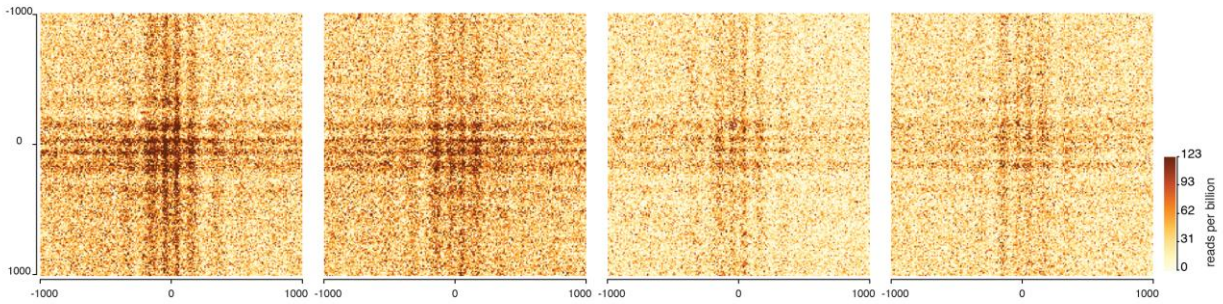


Supplementary Fig. 3. H3K4me3-enriched anchors are engaged in more loops associated with higher transcription rate in both mESC and hTERT-RPE1.
a, b, Violin plots of log-transformed mean NET-seq (a) and RNAPII (b) signal at loops anchors clustered based on their valency in mESC and hTERT-RPE1, respectively.



Supplementary Fig. 4. Bivalent promoter-associated interactions are not observed in hTERT-RPE1.**a**, Rescaled pileup plots of 5′- and 3′-stripes called from the hTERT-RPE1 H3K27me3 dataset, shown on averaged Micro-C-ChIP contact maps for H3K27me3 (top) and H3K4me3 (bottom). **b**,Profile plots of H3K27me3 (left) and H3K4me3 (right) ChIP-seq signal at TSS classified as in Fig. 6b. The data is shown for ±5 kb window around TSS. **c**, On-diagonal pileup plots of TSS clustered based on H3K27me3 and H3K4me3 mean signal (left panel – high H3K27me3 and low H3K4me3, middle – medium H3K27me3 and

low H3K4me3, right – low H3K27me3 and high H3K4me3). The aggregated contact maps around TSS are shown for H3K27me3 (top) and H3K4me3 (bottom) Micro-C-ChIP at ±200 kb window.



Supplementary Fig. 5. CTCF-associated interactions for all four CTCF motif orientations Off-diagonal pileups of CTCF-CTCF loops (at 10-100 kb apart) for the mESC H3K4me3 Micro-C-ChIP dataset at 10 bp resolution stratified by CTCF motif orientations. The signal per heatmap was normalized by number of CTCF-CTCF loops and the color margines are defined by the lowest 5 and the highest 95 percentiles of all four heatmaps.

Supplementary Protocol

Table of Materials

Product	Cat. #	Vendor
1x DPBS w/o Mg^+ and Ca^+	14190144	ThermoFisher
37% Formaldehyde	252549-500ML	Sigma Aldrich
Glycine	A1067.0500	PanReac AppliChem
NEB BSA	B9000S	NEB
Ethylene glycol bis(succinimidyl succinate) (EGS)	21565	ThermoFisher
NaCl	S9888-1KG	Sigma Aldrich
Tris	10708976001	Merck
MgCl2 1 M	AM9530G	Invitrogen
IGEPAL CA-630 (NP-40)	18896-50ML	Sigma Aldrich
Halt Proteinase inhibitor Sigle-use cocktail (100x)	78430	ThermoFisher
EGTA 0.5M pH 8.0	40121266-1	BioWorld
EDTA Ultrapure 0.5M pH 8.0	15575-038	Invitrogen
Tween20	P7949-100ML	Sigma Aldrich
Ultrapure Phenol:Chloroform:Isoamyl Alcohol (PCI)	15593-031	Invitrogen
Micrococcal Nuclease (MNase)	Wortington Biochem	LS004798
QIAquick PCR purification kit	28106	QIAgen
Agarose	1613102	BIO-RAD
Ethidium Bromide	15585-011	Invitrogen
10 U/ml T4 PNK	M0201L	NEB
5 U/ml Klenow Fragment	M0210L	NEB
1 mM Biotin dATP	NU-835-Bio14-S	Jenna Bioscience
1 mM Biotin dCTP	NU-809-BioX-S	Jenna Bioscience
10 mM dTTP	N0443S	NEB
10 mM dGTP	N0442S	NEB
10x T4 DNA Ligase buffer	B0202A	NEB
400 U/ml Ta DNA Ligase	M0202L	NEB
10x NEBuffer #1	B7001S	NEB
100 U/L Exonuclease III	M0206L	NEB
Proteinase K	GoldBio	P-480-1
Ultrapure 10% SDS	15553-035	Invitrogen
Dynabeads MyOne Streptavidin C1	65001	Invitrogen
TE buffer		
NEBNext Ultra II DNA library prep kit for Illumina	E7645L	NEB

NEBNext Multiplex Oligos for Illumina (Dual Index primers)	E7600S	NEB
NEBNext Ultra II Q5 Master mix	M0544S	NEB
SPRIselect size selection beads	B23319	Beckman Coulter
Qubit dsDNA HS Assay kit	Q32854	Invitrogen
Ethanol 96%	20824.365	VWR Chemicals
Gel Loading dye purple (6X)	B7024S	NEB
Quick load purple 1kb plus DNA Ladder	N0550S	NEB
Dimethyl Sulfoxide (DMSO)	D8418-100ML	Sigma Aldrich
Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb	9733s (LOT:14)	Cell Signaling Technology
Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb	9751S (LOT:19)	Cell Signaling Technology
Pierce Protein A Magnetic Beads	88846	ThermoFisher
Triton X-100	85111	ThermoFisher
Sodium deoxycholate	30970-25G	Merck

Micro-C-ChIP protocol

Micro-C-ChIP is a chromosome conformation capture technology that allows to profile histone mark-specific chromatin organization. Here, we combine the high-resolution method Micro-C with histone PTM-specific chromatin immunoprecipitation, a hybrid methodology we call Micro-C-ChIP. The protocol was developed for mouse embryonic stem cells (mESC) and hTERT-immortalized retinal pigment epithelial cells (hTERT-RPE1).

Day 1

1. **Cell culture and dual crosslinking**
   I. Culture cells according to the experimental needs to obtain a minimum of 2 x 10^7 cells. **NOTE**: 5-10 x 10^6 cells are normally used per preparative Micro-C-ChIP, while 1 x 10^6 cells is used for MNase titration (step 2). Since sonication optimization might be needed (step 5), it is recommended to account for that too.
   II. At 70%-80% confluency, aspirate the medium and wash once with DPBS. Trypsinize and pellet cells after quenching trypsin with pre-warmed media.
   III. Discard supernatant, resuspend cells in DPBS and count.

   NOTE: Centrifugation speed and duration as well as trypsinization time need to be adjusted according to a cell type that is used.

   IV. Collect the cells by centrifugation and resuspend them in DPBS at a final concentration of 1 x 10^6 cells/mL (e.g., 1 x 10^7 cells in 10 mL DPBS).

V. For the first crosslinking, add 37% formaldehyde (FA) to a final concentration of 1% to the cell suspension, and incubate the cell suspension for 10 min at RT with rotation (e.g., for the yield of 1 x 10^7 cells resuspended in 10 mL DPBS, add 275 µl of FA).

VI. To quench the reaction, add 2.5 M glycine to a final concentration of 0.25 M and incubate for 5 min at RT with rotation (e.g., if the yield is 1 x 10^7 cells, add 1.142 mL of glycine to the cell suspension).

VII. Pellet the cells at 1,000 x g for 5 min at RT. Discard the supernatant and resuspend the cells in 5-10 mL of DPBS to wash the cells. Repeat the centrifugation step and resuspend the pellet in DPBS to 4 x 10^6 cells/mL (e.g., if the yield is 1 x 10^7 cells, resuspend the cells in 2.5 mL of DPBS).

VIII. For the second crosslinking, add 0.3 M ethylene glycol bis-succinimidyl succinate (EGS) at a final concentration of 3 mM to the cell suspension, and incubate at RT for 40 min with rotation (e.g., for the yield of 1 x 10^7 cells resuspended in 2.5 mL DPBS, add 25 µl of EGS).

NOTE: Equilibrate the EGS to RT for at least 20 min before weighing. Prepare the 0.3 M stock solution of EGS in DMSO (13.6 mg of EGS in 100 µl of DMSO).

IX. To quench the reaction, add 2.5 M glycine to a final concentration of 0.4 M and incubate at RT for 5 min with rotation (e.g., if the yield is 1 x 10^7 cells, add 400 µl of glycine to the cell suspension).

X. Pellet the cells at 1,000 x g for 5 min at RT and discard the supernatant.

XI. Resuspend the pellet in DPBS at 5 x 10^6 cells/mL concentration. Distribute 5-10 x 10^6 cells/tube for preparative libraries and 1 x 10^6 cells/tube for MNase titration.

NOTE: It is recommended to titrate the optimal digestion degree for each cross-linked cell batch. Ideally, collect two to three aliquots of 1 x 10^6 cells for MNase titration (step 2) and two to four aliquots of 5-10 x 10^6 cells for preparative experiments (step 3).

XII. Pellet the cells at 1000 x g for 5 min at RT and remove the supernatant. Snap-freeze the cell pellets in liquid nitrogen, and store them at −80 °C.

Day 2

2. **MNase titration**

I. Thaw one aliquot of previously double cross-linked 1 x 10^6 cells on ice for 10 min and resuspend the cells in 500 µl of DPBS (add 1x BSA if the cells stick to the wall). Incubate the cell suspension on ice for 20 min.

II. Pellet the cells at 10,000 x *g* for 5 min at RT and remove the supernatant.

III. Resuspend the pellet in 500 µl of MB#1 buffer to obtain nuclei.

IV. Pellet the cells at 10,000 x g for 5 min and remove the supernatant. Resuspend the pellet in 200 µl of MB#1 buffer. Split the sample into four tubes (50 µl of each which is the equivalent of 2.5 x 10^5 cells).

V. Dilute the MNase stock (20 U/µl) with 10 mM Tris (pH 7.4) in a series of 1:2, 1:4, 1:4, and 1:4 to achieve concentrations of 10 U/µl, 2.5 U/µl, 0.625 U/µl, and 0.1256 U/µl, respectively (one for each digestion condition).

VI. Add 1 µl of one MNase dilution to one of the four samples, vortex, spin briefly and incubate on a thermomixer at 37 °C for 10 min (800 rpm shaking). Continue with adding 1 µl from the remaining MNase dilutions to the remaining cell aliquots using 10-20 s time intervals.

VII. To stop the MNase digestion, add 200 µl of freshly prepared STOP buffer (150 µl of 10 mM Tris, pH 7.5, 25 µl of 10 % SDS, 25 µl of 20 mg/mL proteinase K, 2 µl of 0.5 M EGTA) to each tube in the same order and with the same time interval that the MNase was added. Incubate at 65 °C for 2 h.

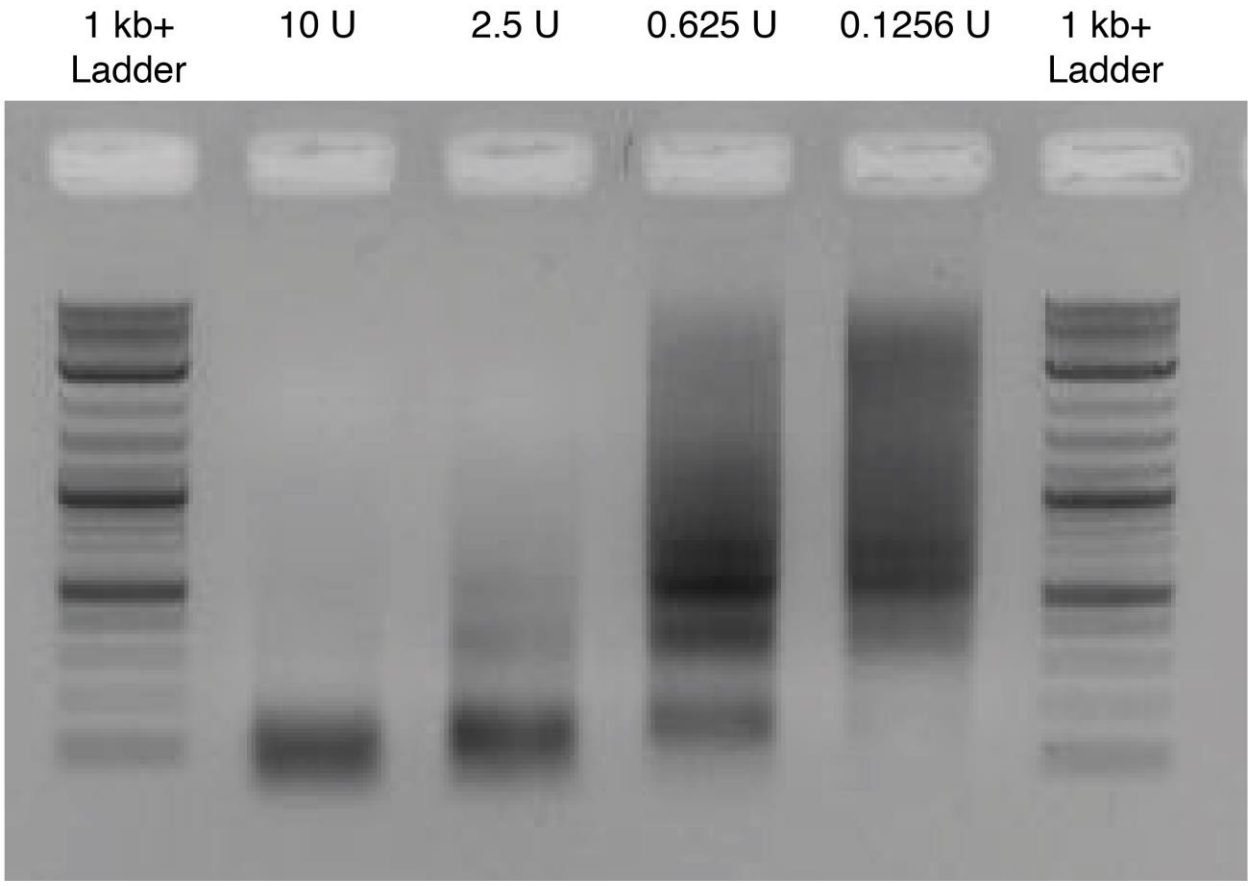
VIII. Add 500 µl of phenol-chloroform-isoamyl alcohol (PCI) to each sample and mix thoroughly by vortexing. Centrifuge at 19,800 x g for 5 min at RT and transfer the upper (aqueous) phase to new tubes (approximately 200 µl/sample).

IX. Purify the DNA with either a commercial DNA purification kit (see Table of Materials) according to the manufacturer's instructions or ethanol precipitation and elute the samples in 12 µl of elution buffer.

X. Add 5 µl of loading dye and run the samples on a 1.5% agarose gel at 120 V for 30-50 min (until properly separated).

XI. Select the best digestion degree for the experiment and continue to the preparative MNase digestion. An optimal digestion degree should have a 70%-90 % mono- to di-nucleosome ratio and little to no subnucleosomal fragments.

NOTE: As can observed from the example of the MNase titration gel, the digestion obtained with MNase in the range 0.625-2.5 U for 2.5 x 10^5 cells (= 2.5-10 U of MNase for 1 x 10^6 cells) is optimal. In this case, the ideal MNase amount is 5 U for 1 x 10^6 cells.



Day 3

3. **Preparative MNase digestion**

I. Thaw previously double cross-linked 5 x 10^6 cell aliquot on ice for 10 min and resuspend in 1 mL of DPBS. Incubate on ice for 20 min (add 1x BSA if the cells stick to the tube wall).

II. Pellet the cells at 10,000 x g for 5 min at RT and discard the supernatant. Resuspend the pellet in 500 µl of MB#1 buffer to obtain nuclei. Repeat the centrifugation step. Resuspend the pellet in 1 mL of MB#1 buffer and make five 200 µl aliquots (equivalent of 10^6 cells per aliquot).

III. Digest the chromatin by adding 1 µl of MNase (typically, 2.5-10 U/µl per 1 x 10^6 cells) to each 200 µl aliquot, based on the MNase titration (step 2). Vortex, spin quickly, and incubate on a thermomixer at 37 °C for 10 min with 800 rpm shaking. Continue adding 1 µl MNase to the remaining cell aliquots using equal time intervals (10-20 s) in between.

IV. To stop the MNase digestion, add1.6 µl of 0.5 M EGTA (final 4 mM) to each aliquot in the same order and with the same time interval that the MNase was added. Incubate on a thermomixer at 65 °C for 10 min with 800 rpm shaking.

V. Pellet the nuclei at 10,000 x g for 5 min at RT and discard the supernatant. Resuspend the pellet in 500 µl of 1x T4 DNA Ligase Buffer.

VI. Pool samples equivalent to an input of 5 x 10^6 cells for further processing.

NOTE: More than 5 x 10^6 cells can be used to increase library complexity, but these samples should be processed in parallel, since the enzymatic conditions are optimized for 5 x 10^6 cells until the immunoprecipitation step (step 5).

VII. Transfer 2-5% of the sample as an input control to control for the MNase digestion level. Add 150 µl of 10 mM Tris, pH 7.4, 25 µl of 10% SDS, and 25 µl of 20 mg/mL proteinase K to this sample and incubate 2h/overnight at 65 °C.

4. **DNA end processing and proximity ligation**

I. Pellet the pooled sample at 10,000 x g for 5 min at 4 °C and discard the supernatant. Resuspend the pellet in 90 µl of freshly prepared Micro-C "Master Mix 1" and incubate on a thermomixer for 15 min at 37 °C with 800 rpm shaking.

II. Add 10 µl of 5 U/µl Klenow Fragment and incubate on a thermomixer for 15 min at 37 °C with 800 rpm shaking.

III. Add 100 µl of freshly prepared Micro-C "Master Mix 2" and incubate on a thermomixer for 45 min at 25 °C with 800 rpm shaking.

IV. Quench the enzymatic reaction by adding EDTA to a final concentration of 30 mM. Incubate on a thermomixer for 20 min at 65 °C with 800 rpm shaking.

V. Pellet the sample at 10,000 x g for 5 min at 4 °C. Discard supernatant. Resuspend the pellet in 500 µl of 1x T4 DNA Ligase Buffer.

VI. Pellet the sample at 10,000 x g for 5 min at 4 °C. Discard supernatant.

VII. Resuspend the pellet in 500 µl of freshly prepared Master Mix 3. Incubate for 2-2.5 hours at RT with slow rotation.

VIII. Pellet the sample at 12,000 x g for 5 min at 4 °C and discard the supernatant. Resuspend the pellet in 200 µl of freshly prepared Micro-C master mix 4 and incubate on a thermomixer for 15 min at 37 °C with 800 rpm shaking.

IX. Transfer 2-5% of the sample as an input control to control for the ligation efficiency. Add 150 µl of 10 mM Tris, pH 7.4, 25 µl of 10% SDS, and 25 µl of 20 mg/mL proteinase K to this sample and incubate 2h/overnight at 65 °C.

5. **Sonication and immunoprecipitation**

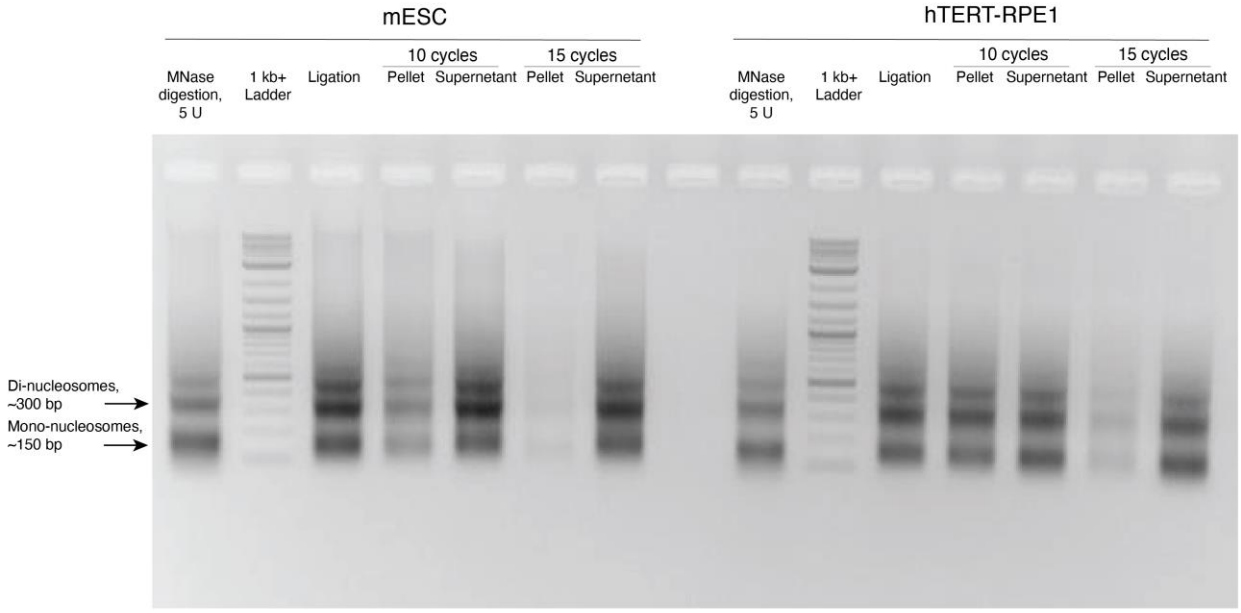
NOTE: The sonication conditions (e.g., sonication cycles, percentage of detergent, sonication buffer volume etc.) can vary depending on a cell type and a sonicator available (here, Bioruptor Plus from Diagenode is used). It is recommended to optimize the sonication procedure for every cell type beforehand. In this example, mouse embryonic stem cells (mESC) and hTERT-immortalized retinal pigment epithelial cells (hTERT-RPE1) are used.

NOTE: When optimizing sonication conditions, we recommend doing it for the standard starting number of cells, namely 5 x 10^6 cells. The solubilized fraction can be used to estimate the DNA yield.

I. Pellet the sample at 12,000x g for 5 min at 4 °C, and discard the supernatant. Resuspend the pellet (equivalent of ~ 5 x 10^6 cells) in 250 µl of freshly prepared Sonication Buffer.

II. Sonicate the sample for 10-15 cycles (sonication cycle: 30 sec ON, 30 sec OFF).

NOTE Since the chromatin is already fragmented, the main purpose of the sonication is to release a high fraction of proximity-ligated di-nucleosomal sized DNA fragments into the soluble fraction. Overshearing can lead to breakage of previously proximity-ligated DNA which might further affect the library quality.



III. Pellet the sonicated sample at 14,000x g for 10 minutes at 4 °C

IV. Transfer the supernatant (~200 µl) to a 2 mL tube.

NOTE: At this stage, it is possible to pull 5 x 10^6 cell-equivalent aliquots, which were processed in parallel beforehand, to increase immunoprecipitation yield and library complexity.

V. Before proceeding to the antibody incubation, transfer 2-5% of the sample as an input control to control for the sonication efficiency. Add 150 µl of 10 mM Tris, pH 7.4, 25 µl of 10% SDS, and 25 µl of 20 mg/mL proteinase K to this sample and incubate 2h/overnight at 65 °C.

VI. Dilute the collected supernatant (and pooled, if needed) 1:4 with ChIP Dilution Buffer (e.g., 800 µl to 200 µl of supernatant).

VII. Add antibody according to the manufacturer's instructions. For H3K27me3 and H3K4me3, antibodies are added at concentration 10 µl of antibody per 10 µg of chromatin (typically 10-20 µl of antibody for 5-10 x 10^6 cell-equivalent sample). Incubate overnight at 4 °C with rotation.

Day 4

VIII. Aliquot 50 µl of Pierce ChIP-grade protein A/G magnetic beads per sample and briefly vortex.
IX. Place tube on magnetic rack and allow beads to separate for approximately 30 sec. Remove supernatant with a pipette.
X. Resuspend beads in 300 µl of ChIP Dilution Buffer per sample, vortex and place on magnetic rack and wait for beads to separate, then remove supernatant.
XI. Wash the beads once again the same way as described above.
XII. Resuspend beads in 50 µl of ChIP Dilution Buffer per sample.
XIII. Add 50 µl of the resuspended beads per one immunoprecipitation reaction. Incubate for 2h at 4 °C with rotation.

6. ChIP washings and DNA elution

NOTE: Wash buffers should be freshly prepared and kept on ice.

I. After incubation with the beads is complete, briefly spin the samples and place on magnetic rack and allow beads to separate for approximately 30 sec.
II. Wash beads for 5 min on a rotating platform at RT with 1 mL of each of the following buffers. Following washing place tube on magnetic rack, remove supernatant, and add the next wash buffer in the following order:
    - Low-salt wash buffer - two washes
    - High-salt wash buffer- two washes
    - LiCl wash buffer - one wash
    - TE buffer - two washes
III. After the last washing step is complete, add 250 µl of ChIP Elution Buffer per sample and incubate at 65 °C 4h/overnight with intermittent mixing.
IV. Vortex and spin the sample briefly.
V. Add 500 µl of PCI to each sample as well as input control samples (MNase digestion control (from step 3), ligation control (from step 4) and sonication control (from step 5)), and vortex thoroughly. Spin at 19,800 x g for 5 min at RT and transfer the upper (aqueous) phase to new tubes (approximately 200 µl/sample).
VI. Purify DNA by ethanol extraction: add 2.5X volume of 100% ethanol that has been pre-chilled to –20 °C. Incubate for 30 minutes at –20 °C. Spin for 30 min at 19,800 at 4 °C. Wash wallet adding 70% ethanol. Spin for 5 min at 19,800 at 4 °C. Remove supernatant and use a P10 to remove all traces of ethanol without disturbing the pellet. Air-dry pellet at RT until no ethanol is visible. Resuspend pellet in 20 µl of 0.1x TE buffer. Incubate for 30 minutes at 37 °C.
VII. Determine DNA concentration using the Qubit Fluorometer with the dsDNA HS Assay Kit according to the manufacturer's protocol.
VIII. After measuring DNA, bring the sample volume to 150 µl adding 0.1x TE buffer.

IX. Run the input control samples on a 1.5% agarose gel at 120 V for 30-50 min (until properly separated).

Day 5

7. **Streptavidin pull-down and on-bead library preparation**

I. Aliquot 10 µl of streptavidin beads per sample into a reaction tube.
II. Place tube on magnetic rack and allow beads to separate for approximately 30 sec. Remove supernatant.
III. Resuspend the beads in 500 µl of 1x TBW, vortex and place on magnetic rack and wait for beads to separate, then remove supernatant.
IV. Wash the beads once again the same way as described above.
V. Resuspend the beads in 150 µl of 2x B&W buffer per sample.
VI. Add 150 µl of the beads to 150 µl of the sample. Incubate for 20 min at RT with rotation.
VII. Place the tubes on magnetic rack and wait until the solution clears. Remove the supernatant, resuspend the beads in 300 µl of 1x TBW, vortex and place on magnetic rack and wait for beads to separate, then remove supernatant. Repeat this step.
VIII. Resuspend the beads in 100 µl of 0.1x TE, vortex and place on magnetic rack and wait for beads to separate, then remove supernatant.
IX. Resuspend the beads in 50 µl of 0.1x TE and transfer them to PCR tubes.
NOTE: The volume of 0.1x TE used corresponds to the input volume of the DNA sequencing library preparation kit (see Table of Materials) used in this protocol. For a different kit, you might need to adjust the volume accordingly.
X. Perform the DNA manipulation steps of the sequencing library preparation kit (here, NEBNext Ultra II DNA library prep kit for Illumina) (see Table of Materials) according to the manufacturer's protocol. These steps include:

- DNA blunting;
- A-tailing;
- adaptor ligation;
- U excision.

Follow the manufacturer's instructions from step 1 to step 2.6 in the NEBNext kit protocol diluting adaptors based on how much DNA you have (typically, 1:9), and do not consider −20 °C storage after step 2.6.

Note: Because the sample remains attached to beads, all purification and size selections steps were omitted.

XI. After adaptor ligation, remove the supernatant. Resuspend the beads in 150 µl of 1x TBW, vortex and place on magnetic rack and wait for beads to separate, then remove supernatant. Repeat this step once.
XII. Resuspend the beads in 100 µl of 0.1x TE, vortex and place on magnetic rack and wait for beads to separate, then remove supernatant.
XIII. Resuspend the beads in 20 µl of 0.1x TE.

8. **Sequencing library amplification and post-PCR clean-up**

I. Add PCR master mix (64 µl of H2O, 100 µl of Q5 high-fidelity DNA polymerase, 8 µl of i5 primer, 8 µl of i7 primer) to the 20 µl of the streptavidin-biotin-DNA sample. Mix the reaction well by pipetting up and down. Split the reaction mixture into 100 µl aliquots.

NOTE: The primers used here are Dual Index primers from NEBNext Multiplex Oligos for Illumina (see Table of Materials). Primers used need to be compatible with the library preparation kit used.

II. Perform PCR according to the manufacturer's instructions for Q5 high-fidelity DNA polymerase:

Denaturation	98°C	30 sec
12-16 cycles	98°C 65°C 65°C	10 sec 30 sec 45 sec
Extension	65°C	5 min
	4°C	Hold

III. Transfer the samples to a low-retention 1.5 mL tube pooling back samples split into 100 µl aliquots.

IV. Place the tube on a magnetic rack and wait until the solution clears. Move the supernatant containing the PCR-amplified DNA to a new 1.5 mL tube.

V. For each 200 µl of post-PCR sample, add 180 µl of SPRI beads (see Table of Materials) (ratio of 1:0.9) and pipet up and down until thoroughly resuspended. Allow the DNA to bind to the beads incubating for 20 min at RT.

VI. Place the tubes on magnetic rack and wait until the solution clears. Remove the supernatant.

VII. Add 200 µl of freshly prepared 80% ethanol. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Repeat this step once.

VIII. Remove residual ethanol without disturbing the beads.

IX. Air-dry the bead until no ethanol is visible. Resuspend the beads in 21 µl of 0.1x TE buffer or water and vortex well. Incubate for 2 minutes to allow DNA to elute from the beads.

X. Place the tube back on the magnetic rack and wait until the solution is clear. Transfer the supernatant to a new tube.

XI. Determine DNA concentration using the Qubit Fluorometer with the dsDNA HS Assay Kit according to the manufacturer's protocol.

XII. Determine the library fragment distribution using Fragment Analyzer or a similar system.

NOTE: If adaptor dimers are observed, indicating low library complexity, it is highly recommended to quality control the sample with low input sequencing.

9.DNA sequencing

I. Sequence the Micro-C-ChIP library with paired-end sequencing according to the sequencing provider's requirements.

NOTE: Typically, the samples are sequenced on a platform in paired-end mode with 50 bp per read.

II. To assess the quality of the library, perform low input sequencing with 1 x 10^7 reads per sample.

Buffer List

MB #1

Components	Final concentration
NaCl	50 mM
Tris-HCl, pH 7.5	10 mM
$MgCl_2$	5 mM
$CaCl_2$	1 mM

Freshly completed with 0.2 % IGEPAL CA-630 (NP-40) and 1X protease inhibitors.

Master Mix 1

Components	1x
10x T4 DNA Ligase buffer	10 µl
10 U/ml T4 PNK	5 µl
H2O	75 µl

Master Mix 2

Components	1x
1 mM Biotin-dATP	10 µl
1 mM Biotin-dCTP	10 µl
10 mM mix of dTTP and dGTP	1 µl
10x T4 DNA Ligase buffer	5 µl
200X BSA	0.25 µl
H2O	23.75 µl

Master Mix 3

Components	1x
10x T4 DNA Ligase buffer	50 µl
200X BSA	2.5 µl
400 U/ml Ta DNA Ligase	25 µl
H2O	422.5 µl

Master Mix 4

Components	1x
10x NEBuffer 1.1	50 µl
100 U/L Exonuclease III	25 µl
H_2O	422.5 µl

Sonication buffer

Components	Final concentration
NaCl	50 mM
Tris-HCl, pH 7.5	10 mM
$MgCl_2$	5 mM
EDTA	2 mM

Freshly completed with 0.1% IGEPAL CA-630 (NP-40), 0.5% SDS and 1X protease inhibitors.

ChIP Dilution Buffer

Components	Final concentration
NaCl	167 mM
Tris-HCl, pH 7.5	16.7 mM
EDTA	1.2 mM

Freshly completed with 1.1% Triton X-100 and 1X protease inhibitors.

Low-salt wash buffer

Components	Final concentration
NaCl	150 mM
Tris-HCl, pH 8	20 mM
EDTA	2 mM

Freshly completed with 1% Triton X-100, 0.1% SDS and 1X protease inhibitors.

High-salt wash buffer

Components	Final concentration

NaCl	500 mM
Tris-HCl, pH 8	20 mM
EDTA	2 mM

Freshly completed with 1% Triton X-100, 0.1% SDS and 1X protease inhibitors.

LiCl wash buffer

Components	Final concentration
LiCl	250 mM
Tris-HCl, pH 8	10 mM
EDTA	1 mM

Freshly completed with 1% Sodium deoxycholate, 1% IGEPAL CA-630 (NP-40) and 1X protease inhibitors.

1xTE

Components	Final concentration
Tris-HCl, pH 8	10 mM
EDTA	1 mM

ChIP Elution Buffer

Components	Final concentration
NaCl	300 mM
Tris-HCl, pH 8	20 mM
EDTA	10 mM
EGTA	5 mM

Freshly completed with 1 mg/ml Proteinase K and 1% SDS.

TBW

Components	Final concentration
NaCl	1000 mM
Tris-HCl, pH 7.5	5 mM
EDTA	0.5 mM
Tween 20	0.05%

2X B&W buffer

Components	Final concentration

NaCl	2000 mM
Tris-HCl, pH 7.5	10 mM
EDTA	1 mM